

# THE PHYLOGENY OF SELENIA (BRASSICACEAE) INFERRED FROM CHLOROPLAST AND NUCLEAR SEQUENCE DATA

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## ABSTRACT

*Selenia* Nutt. (Brassicaceae) is a North American genus of five species distributed from the central and southwestern U.S.A. to northern Mexico. While the basic taxonomy of this group has been well established, very little is known about the biology of individual species or the phylogenetic relationships among them. In this study, DNA sequence variation from the nuclear internal transcribed spacer and four non-coding chloroplast regions (the *trnL* intron; and the *petA-psbJ*, *trnQ-rps16*, and *trnS<sup>GCU</sup>-trnG<sup>UUC</sup>* intergenic spacers) was used to reconstruct the generic phylogeny. Results of parsimony and Bayesian analyses strongly supported the monophyly of both the genus and each individual species, and a completely resolved intra-generic phylogeny. The single instance of conflict between the nuclear and chloroplast topologies indicated historical hybridization between *Selenia grandis* and *Selenia mexicana*. The phylogenetic distinctiveness of *S. mexicana*, known only from a few collections in Nuevo León, combined with the relative lack of collections from Mexico suggested that additional diversity awaits discovery in this group.

## RESUMEN

*Selenia* Nutt. (Brassicaceae) es un género norteamericano con cinco especies que se distribuyen desde el centro y sudoeste de Estados Unidos hasta el norte de México. Aunque la taxonomía básica de este grupo está bien establecida, se sabe muy poco sobre la biología de las especies o las relaciones filogenéticas entre ellas. En este estudio para la reconstrucción de la filogenia del género se emplearon cambios en las secuencias del espaciador de transcripción interna específico del ADN ribosomal (ADNr) y cuatro regiones no codificantes del ADN plastidial (el intrón *trnL* y los espaciadores intergénicos *petA-psbJ*, *trnQ-rps16*, y *trnS<sup>GCU</sup>-trnG<sup>UUC</sup>*). Los análisis de máxima parsimonia y bayesianos apoyan sólidamente la monofilia tanto del género, como de cada especie por separado, en una filogenia intragenérica completamente resuelta. El conflicto entre las topologías obtenidas con ADN nuclear y cloroplástico indican hibridación histórica entre *Selenia grandis* y *Selenia mexicana*. La distinción filogenética de *S. mexicana*, apenas conocida de unas pocas localidades en Nuevo León, junto con las pocas colecciones mexicanas sugiere que se espera descubrir más diversidad en este grupo.

## INTRODUCTION

*Selenia* Nutt. (Brassicaceae) is a distinctive genus of five species distributed from the central and southwestern U.S.A. to northern Mexico (Fig. 1). *Selenia* species are small (<50 cm tall), spring flowering, herbaceous annuals found on a wide range of often seasonally wet habitats from sandstone glades (*S. aurea* Nutt.) to limestone hills (*S. dissecta* Torr. & A. Gray) to alluvial soils (*S. grandis* R.F. Martin) (Rollins 1993). *Selenia* can be easily distinguished from all other genera of the tribe Cardamineae by a combination of an annual habit, fully bracteate inflorescences, yellow flowers, silicles with distinct style, and biseriate, broadly winged seeds. Although *S. aurea* and *S. dissecta* can be found in multiple states, known from 57 and 14 counties, respectively, *S. grandis* and *S. jonesii* Cory are endemic respectively to southern and western Texas in the U.S.A. *Selenia mexicana* Standl. is known only from the Mexican states of Coahuila and Nuevo León, although the individual ranges of this species and of *S. dissecta*, *S. jonesii*, and *S. grandis* will surely expand following additional fieldwork in northern Mexico. This biogeographic uncertainty is representative of a basic lack of knowledge regarding *Selenia*, and little to no information exists concerning the reproductive biology, ecology, and phylogenetic relationships within this distinctive group (Al-Shehbaz 1988).

Although both morphological and biogeographic patterns within *Selenia* suggest certain null phylogenetic hypotheses, no study has addressed these evolutionary relationships. *Selenia aurea* is morphologically divergent from its congeners, with unappendaged sepals, pinnate (vs. bipinnate) leaves, and relatively long (>5 mm) styles. These features and its disjunct range (Fig. 1) suggest an isolated phylogenetic position for

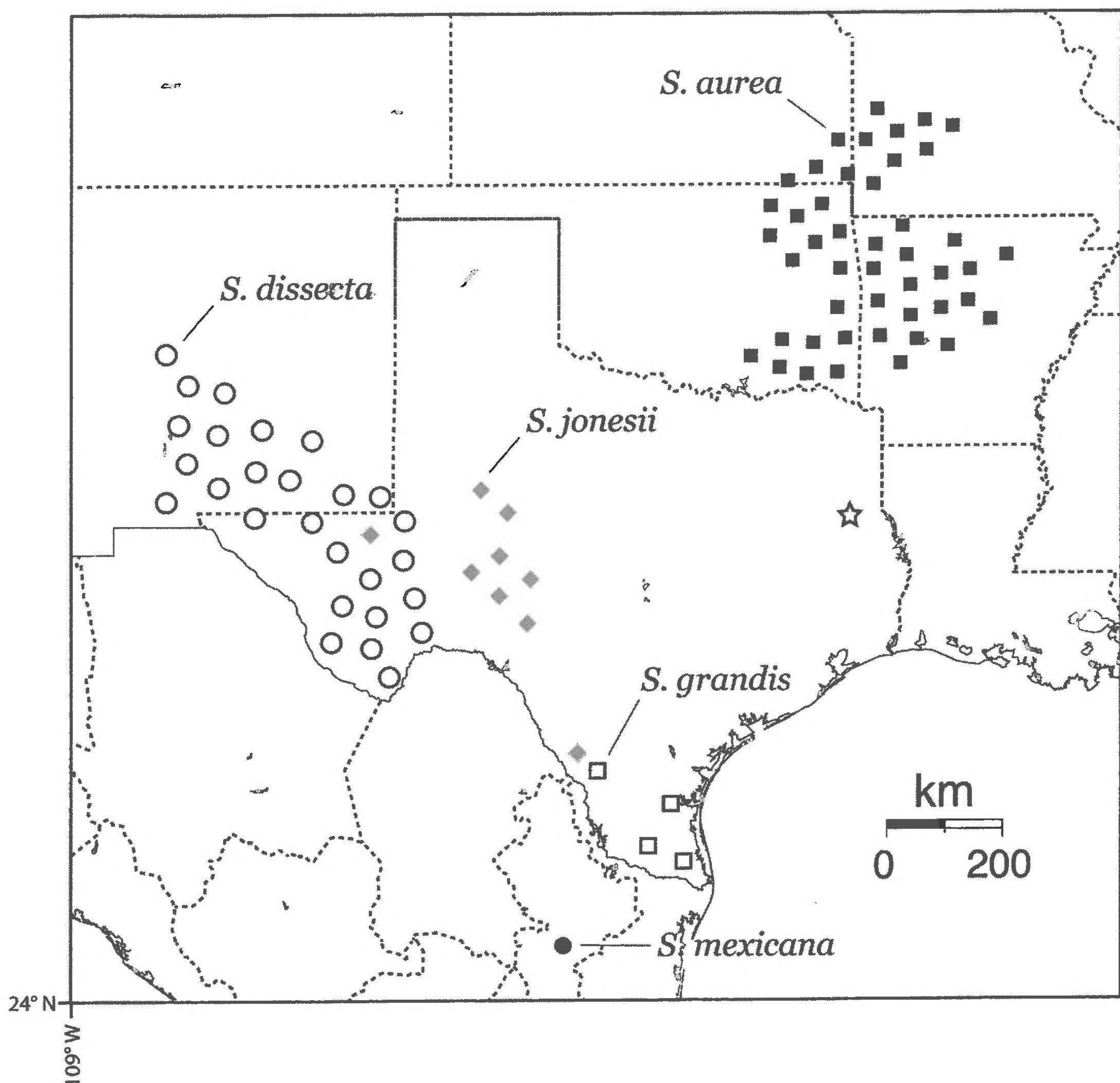


FIG. 1. The geographic distribution of *Selenia* species. All ranges are generalized and approximate, particularly that of *S. mexicana*. The potential range of *S. aperta* (see text) is noted with a star. The base map was created using software available at: [http://www.aquarius.geomar.de/omc/make\\_map.html](http://www.aquarius.geomar.de/omc/make_map.html)

this species. Within the remaining species, several characters, including possession of a horn-like (vs. pouch-like) sepal appendage and relatively long (>8 mm) sepals and anthers (>2.5 mm) suggest that *S. dissecta*, *S. grandis*, and *S. mexicana* form a natural group. These patterns of shared character variation, the morphological cohesiveness of individual species (Al-Shehbaz 1988), and the small size of the genus suggest that reconstructing the evolutionary relationships within this distinctive North American taxon will be tractable. This study aims to resolve the phylogenetic relationships between the five recognized species of *Selenia* using both chloroplast and nuclear DNA sequence variation.

#### MATERIALS AND METHODS

##### TAXON SAMPLING

Sample information appears in Appendix 1. Ten *Selenia* samples were analyzed, including two samples from each of the five species recognized in perhaps the most focused examination of the genus (Martin 1940). Three taxa that have been recognized by certain authors were not included, *S. jonesii* var. *obovata* Rollins, *S.*

*aperta* (S. Watson) Small, and *S. oinosepala* Steyermark. The first taxon is known only from the type collection (Rollins 1993), and the distinguishing character state (obovate fruits) has been considered to be an artifact of pressing the inflated fruits (Al-Shehbaz in ms.). Martin (1940) provided a detailed discussion of the lack of distinctiveness of both *S. aperta* and *S. oinosepala*, although both taxa warrant additional study (see discussion). *Leavenworthia* Torr. has been shown to be sister to *Selenia*, and the monotypic genus *Planodes* Greene has been shown, along with *Barbarea* R. Br., to be sister to the *Selenia/Leavenworthia* clade (Beilstein et al. 2006). Two *Leavenworthia* samples [*L. uniflora* (Michx.) Britton and *L. alabamica* Rollins] and a *P. virginicum* (L.) Greene sample were therefore used as outgroups. Nine of ten *Selenia* samples were obtained from herbarium material, and collections made as early as 1958 (see Appendix 1) yielded successful amplifications and sequences.

### Molecular Methods

Extractions were performed with either a Qiagen (Qiagen, Valencia, CA) DNeasy Plant Mini Kit or a Viogene (Viogene U.S.A., Sunnyvale, CA) extraction kit. The nuclear internal transcribed spacer (ITS) region was amplified using the primers "ITS 1" (White et al. 1990) and either "ITS 4" (White et al. 1990) or "ITS2-26S.4" (Rauscher 2002). The *trnL*<sup>UAA</sup> intron was amplified using the primers "C" and "D" (Taberlet et al. 1991). A portion of the chloroplast *trnS*<sup>GCU</sup>-*trnG*<sup>UUC</sup> intergenic spacer was amplified with the primers "1F" and "1R" (Säll et al. 2003). A portion of the chloroplast *petA-psbJ* intergenic spacer was amplified with the primers "5F" and "5R" (Säll et al. 2003). A portion of the chloroplast *trnQ*<sup>UUG</sup>-*rps16* intergenic spacer was amplified with the primers "trnQ<sup>UUG</sup>" and "rpS16x1" (Shaw et al. 2007). All reactions were performed under standard conditions. Products were visualized and purified via agarose gel electrophoresis with a Viogene gel extraction kit. Products were dye-labeled using a Big Dye Terminator Kit (Applied Biosystems, Foster City, CA), and analyzed on either a MJ Research BaseStation (MJ Research, Waltham, MA) or an Applied Biosystems 3130xl Genetic Analyzer. All sequences have been deposited in the EMBL nucleotide sequence database (Appendix 1).

### Phylogenetic Analyses

The ITS and combined chloroplast (*trnL*, *trnS-trnG*, *petA-psbJ*, and *trnQ-rps16*) datasets were analyzed separately. Sequences were manually aligned in Se-Al (Rambaut 2002) and the aligned matrix was exported as a NEXUS file. All insertion/deletion (indel) events, both autapomorphic and synapomorphic, were scored except in the case of nucleotide repeats resulting in more than two indel character states (which were viewed as likely homoplasious), or in regions of uncertain alignment. In the case of overlapping indel events, the "simple gap coding" method of Simmons and Ochoterena (2000) was used. All positions involved in indels, or situated in regions of uncertain alignment were deleted prior to analysis, and indel events were coded as additional 1/0 characters and added to the end of the NEXUS file. Positions exhibiting poor sequence or additivity (multiple peaks presumably due to the presence of divergent ITS sequences in a single individual) were coded as ambiguous. For each dataset a heuristic maximum parsimony search with 100 random addition replicates was performed using PAUP\* 4.0b10 (Swofford 2002) with the following parameters: starting trees obtained by stepwise addition, TBR branch swapping, "MulTrees" turned on, and steepest descent not in effect. Ten thousand bootstrap replicates were conducted with PAUP\* 4.0b10 in order to obtain bootstrap support (BS). The best-fitting model of sequence evolution for each DNA region (indels and poorly aligned regions excluded) was identified using the Akaike Information Criterion in Modeltest 3.06 (Posada & Crandall 1998), and a Bayesian Markov Chain Monte Carlo analysis was performed on each dataset in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The combined chloroplast data were analyzed as a partitioned dataset, with the best-fitting model of sequence evolution for each separate region assigned to the corresponding partition (see Table 1). For both the ITS and combined chloroplast analyses, the indel characters were assigned the binary model of character evolution (Nst=1, Coding=Variable) as recommended in the MrBayes documentation. All Bayesian analyses comprised four independent runs, with four chains (one cold and three heated). Flat priors were used, with the exception of the rate prior that was set to allow rates to vary among partitions. Chains were run for 5 million generations, and trees were sampled every 1000 generations. Stationarity was evaluated by examining the standard deviation of split frequencies among runs and

TABLE 1. Relative phylogenetic information and model of sequence evolution chosen in each of the five DNA regions analyzed. <sup>a</sup>Includes one inversion event. <sup>b</sup>Only a subset of the models evaluated by Modeltest are available for implementation in MrBayes.

| Sequence characteristic                                | ITS       | trnL    | petA-psbJ | trnS-trnG | trnQ-rps16                       |
|--|-----------|---------|-----------|-----------|----------------------------------|
| Aligned length (bp)                                    | 520       | 578     | 397       | 301       | 523                              |
| Analyzed characters, including indels                  | 516       | 493     | 327       | 286       | 470                              |
| Variable characters, including indels (%)              | 107 (21%) | 23 (5%) | 31 (9%)   | 13 (5%)   | 35 (7%)                          |
| Parsimony informative characters, including indels (%) | 92 (18%)  | 9 (2%)  | 8 (2%)    | 3 (1%)    | 12 (3%)                          |
| Number of indels (parsimony informative)               | 3 (2)     | 7 (4)   | 8 (3)     | 3 (0)     | 9 <sup>a</sup> (4 <sup>a</sup> ) |
| substitution model selected by Modeltest               | TVMef+I   | K81uf+I | K81uf     | K81uf+I   | K81uf                            |
| substitution model implemented in MrBayes <sup>b</sup> | GTR+I     | GTR+I   | GTR       | GTR+I     | GTR                              |

by plotting the log likelihood values from each run using Tracer 1.4 (Rambaut & Drummond 2007). These diagnostics indicated that runs reached stationarity quickly (within 100,000 generations) and I conservatively excluded the first 500,000 generations before obtaining a consensus phylogeny and clade posterior probabilities (PP).

## RESULTS

Details regarding the length, variability, and model of sequence evolution chosen for each gene region are presented in Table 1. The analyzed ITS matrix of 516 characters yielded 107 (21%) variable and 92 (18%) parsimony-informative characters. The matrix contained 10 (0.1%) cells coded as either missing or ambiguous. Additivity, indicated by multiple peaks at a single nucleotide position, was limited to three samples. Two samples (*S. aurea* sample 1 and *S. mexicana* sample 2) exhibited multiple peaks at one position each, while *S. jonesii* sample 2 exhibited multiple peaks at four positions. At each of these four *S. jonesii* positions one of the two inferred nucleotides matched that from the other *S. jonesii* sample, with the other nucleotide a symplesiomorphy, typically observed in several congeners and the outgroup. Two of the three insertion/deletion events were parsimony-informative. Each of the 100 random addition replicate parsimony searches using the ITS dataset recovered the same island of five most parsimonious trees (MPTs) (length = 141, consistency index = 0.86, retention index = 0.91). One of the five MPTs, along with bootstrap percentages and Bayesian posterior probabilities, is shown in Figure 2a. The ITS data provided low support for the monophyly of *Selenia* (0.60 PP, 38% BS), but strong support (1.0 PP, 100% BS) for a “core *Selenia*” clade comprising *S. jonesii*, *S. grandis*, *S. dissecta*, and *S. mexicana*. The ITS data also provided strong support (0.97–1.0 PP) for the sister relationship of each pair of conspecific samples. Certain chloroplast regions failed to amplify in four samples (the *trnQ*<sup>UUG</sup>–*rps16* intergenic spacer in *S. aurea* sample 2, *S. mexicana* sample 2, and the *L. alabamica* sample and the *trnS*<sup>GCU</sup>–*trnG*<sup>UUC</sup> intergenic spacer in *S. dissecta* sample 2). These samples were excluded from the combined chloroplast analysis. The analyzed chloroplast matrix of 1576 characters yielded 102 (6%) variable and 32 (2%) parsimony-informative characters. The chloroplast data matrix contained 29 (0.2%) cells coded as either missing or ambiguous. Eleven of the 27 insertion/deletion events were parsimony-informative. Each of the 100 random addition replicate parsimony searches using the combined chloroplast dataset recovered the same MPT (length = 112, CI = 0.95, RI = 0.88). The MPT, along with bootstrap percentages and Bayesian posterior probabilities, is shown in Figure 2b. Unlike the ITS data, which provided minimal support for the monophyly of *Selenia*, the combined chloroplast dataset strongly indicated such a relationship (1.0 PP, 99% BS). Strong support was also provided for the monophyly of “core *Selenia*” (1.0 PP, 100% BS), and two additional clades nested within this group. Although the four-locus chloroplast dataset was only able to assess the sister relationships of each pair of *S. jonesii* (1.0 PP, 100% BS) and *S. grandis* (1.0 PP, 95% BS) samples, a dataset including only *trnL* intron and *petA-psbJ* intergenic spacer sequences for all 10 *Selenia* samples provided strong support for the sister relationship of each pair of conspecific samples:




FIG. 2. A. One of five MPTs resulting from analysis of the ITS dataset. B. The single MPT resulting from analysis of the four-region chloroplast dataset (*trnL* intron; *trnQ-rps16*, *trnS<sup>GCU</sup>-trnG<sup>UUC</sup>*, and *petA-psbJ* intergenic spacers). Support values appear at each node (Bayesian posterior probabilities/bootstrap percentage). The node marked with an asterisk collapses in the ITS strict consensus tree. The trees were drawn using FigTree 1.1 (Rambaut 2008).

*S. aurea* (1.0 PP, 100% BS), *S. dissecta* (1.0 PP, 90% BS), and *S. mexicana* (1.0 PP, 86% BS). The only conflict between the chloroplast and nuclear topologies involved the placement of *S. mexicana*, which was sister to *S. dissecta* in the nuclear topology and sister to *S. grandis* in the chloroplast topology.

## DISCUSSION

### ***Selenia* phylogeny**

In general the nuclear and chloroplast datasets provide well-resolved and strongly supported phylogenetic reconstructions that are not only congruent with each other (Fig. 2), but with patterns of shared morphological character states. The core *Selenia* clade comprising *S. jonesii*, *S. grandis*, *S. dissecta*, and *S. mexicana* is distinguished by sepals bearing a dorsal appendage, bipinnate (vs. pinnate) leaves, and relatively short (<6 mm) styles. A more exclusive clade comprising *S. grandis*, *S. dissecta*, and *S. mexicana* is distinguished by possession of a horn-like (vs. pouch-like in *S. jonesii*) sepal appendage and relatively long (>8 mm) sepals and anthers (>2.5 mm). Interestingly, even though the *Selenia* key of Martin (1940) is artificial and therefore doesn't necessarily imply relatedness, if it is viewed as a bifurcating tree it perfectly matches the ITS topology presented in Figure 2a. The data clearly support the recognition of *S. mexicana* as distinct from *S. dissecta* (see below), suggesting that additional diversity is yet to be documented in this group. The three taxa not analyzed in this study (*S. jonesii* var. *obovata*, *S. aperta*, *S. oinosepala*) should therefore be subject to future molecular and morphological evaluation. *Selenia aperta* is a particularly intriguing case. This taxon was originally described as a variety of *S. aurea* by Watson (1895) based on material from San Augustine County, Texas, which exhibited broadly inflated silicles, a reduced septum, and a relatively long style. The variety was later given species status by Small (1903). Martin (1940) failed to locate the type material but examined both potential types and other specimens exhibiting these character states. He observed the variation described by Watson but found both variation among individuals from a single collection and no specimens that exhibited the full complement of characters. This variation, the potential disjunct range of *S. aperta* (noted in Fig. 1), and the chromosome number variation noted by Rollins and Rüdenberg (1977) all suggest that additional lineages remain to be identified within *S. aurea*.

### **Potential Hybridization and Chloroplast Capture**

The only incongruence between the two topologies is the placement of *S. mexicana*, and morphological and biogeographical evidence suggest that the nuclear placement (as sister to *S. dissecta*) is correct and that the anomalous placement of *S. mexicana* by the chloroplast data is due to historical hybridization between *S. mexicana* and *S. grandis* followed by chloroplast capture. Chloroplast capture has been well documented empirically (Rieseberg & Soltis 1991), and appears to be possible under a range of biologically realistic situations (Tsitrone et al. 2003; Chan & Levin 2005). From a morphological standpoint, *S. dissecta* and *S. mexicana* are difficult to distinguish, with the latter exhibiting shorter (<2.5 vs. >3.5 mm) styles and spongy (vs. winged) seed margins, and recent workers (Al-Shehbaz 1988; Rollins 1993) have considered it a synonym of *S. dissecta*. Although the ranges of most *Selenia* taxa are poorly known, the existing biogeographic data also lend support to the proposed hybridization scenario, as known populations of *S. mexicana* are approximately 250 km closer to known populations of *S. grandis* than they are to populations of *S. dissecta* (Fig. 1). This evidence notwithstanding, *S. mexicana* is by far the most poorly known *Selenia* species, and additional cytological, genetic, and field studies are needed to thoroughly test this hypothesized gene flow. *Selenia dissecta* has been reported to be  $2n = 14$ , while *S. grandis* is known to be  $2n = 24$  (Warwick & Al-Shehbaz 2006). The currently unknown chromosome number of *S. mexicana* will therefore reveal if the proposed hybridization event was via a polyploid or homoploid pathway. Evidence of additivity in the *S. grandis* or *S. mexicana* ITS sequences was limited to a single position in *S. mexicana* sample 2, indicating that any heterospecific ITS repeats have been largely eliminated due to backcrossing to conspecifics (the homoploid scenario) or concerted evolution (Franzke & Mummenhoff 1999). Particularly in a homoploid hybridization scenario, sequencing both individual cloned ITS sequences and other nuclear loci in an expanded sample set from across both species' ranges will reveal the extent of proposed introgression. Three individuals exhibiting

*S. mexicana* morphology (short styles in particular) were discovered in a recent survey of specimens from six major herbaria (BRIT, GH, MO, NY, TEX-LL, and US), all from within 100 km of the type locality of Galeana, Nuevo León (Standley 1937). Unfortunately, these collections contain few specimens from Mexico, and additional fieldwork is clearly needed. As noted in the introduction, the range of *S. mexicana* is but one of many aspects of *Selenia* species that are poorly documented. Future work, including expanded sampling of all proposed *Selenia* taxa, is therefore needed to understand the biology of this genus, information that can now be placed in an evolutionary context.

#### APPENDIX 1

**Sample information.** Taxon (sample number), voucher, year of collection (if herbarium material): country, state, county (if applicable), ITS EMBL, *trnL* EMBL, *trnS-trnG* EMBL, *petA-psbJ* EMBL, *trnQ-rps16* EMBL.

*Leavenworthia alabamica* Rollins, Beck 486 (MO)—U.S.A. ALABAMA. Franklin Co.: FM957596, FM957609, FM986404, FM986416, none

*Leavenworthia uniflora* (Michx.) Britton, Beck 516 (MO)—U.S.A. ALABAMA. Morgan Co.: FM957595, FM957608, FM986403, FM986415, FM986429

*Planodes virginicum* (L.) Greene, Al-Shehbaz s.n. (MO)—U.S.A. MISSOURI: FM957594, FM957607, FM986402, FM986414, FM986428

*Selenia aurea* Nutt. (1), Beck 774 (MO)—U.S.A. MISSOURI. St. Clair Co.: FM957598, FM957611, FM986406, FM986418, FM986427

*Selenia aurea* Nutt. (2), Stephens 29996 (GH) 1969—U.S.A. OKLAHOMA. Nowata Co.: FM957597, FM957610, FM986405, FM986417, none

*Selenia dissecta* Torr. & A. Gray (1), Worthington 11630 (NY) 1984—MEXICO. Chihuahua, FM957600, FM957613, FM986407, FM986420, FM986431

*Selenia dissecta* Torr. & A. Gray (2), Correll 38395 (TEX-LL) 1970—U.S.A. TEXAS. Culberson Co.: FM957599, FM957612, none, FM986419, FM986430

*Selenia grandis* R.F. Martin (1), Turner 4323 (TEX-LL) 1958—U.S.A. TEXAS. Nueces Co.: FM957603, FM957616, FM986410, FM986423, FM986435

*Selenia grandis* R.F. Martin (2), Correll 36762 (TEX-LL) 1969—U.S.A. TEXAS. Hidalgo Co.: FM957604, FM957617, FM986411, FM986424, FM986434

*Selenia jonesii* Cory (1), Lundell & Lundell 16958 (GH) 1961—U.S.A. TEXAS. Dawson Co.: FM957601, FM957614, FM986408, FM986421, FM986432

*Selenia jonesii* Cory (2), Mahler 8846 (GH) 1981—U.S.A. TEXAS. Reagan Co.: FM957602, FM957615, FM986409, FM986422, FM986433

*Selenia mexicana* Standl. (1), Hinton 27036 (TEX-LL) 1997—MEXICO. Nuevo León: FM957605, FM957618, FM986412, FM986425, FM986436

*Selenia mexicana* Standl. (2), Crutchfield & Johnston 5855 (GH) 1960—MEXICO. Nuevo León: FM957606, FM957619, FM986413, FM986426, none

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